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Identification of Genes Encoding the Folate- and Thiamine-Binding Membrane Proteins in Firmicutes

Eudes, Aymerick; Erkens, Guus B.; Slotboom, Dirk J.; Rodionov, Dmitry A.; Naponelli, Valeria; Hanson, Andrew D.

Published in:
Journal of Bacteriology

DOI:
[10.1128/JB.01070-08](https://doi.org/10.1128/JB.01070-08)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2008

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Citation for published version (APA):

Eudes, A., Erkens, G. B., Slotboom, D. J., Rodionov, D. A., Naponelli, V., & Hanson, A. D. (2008). Identification of Genes Encoding the Folate- and Thiamine-Binding Membrane Proteins in Firmicutes. *Journal of Bacteriology*, 190(22), 7591-7594. <https://doi.org/10.1128/JB.01070-08>

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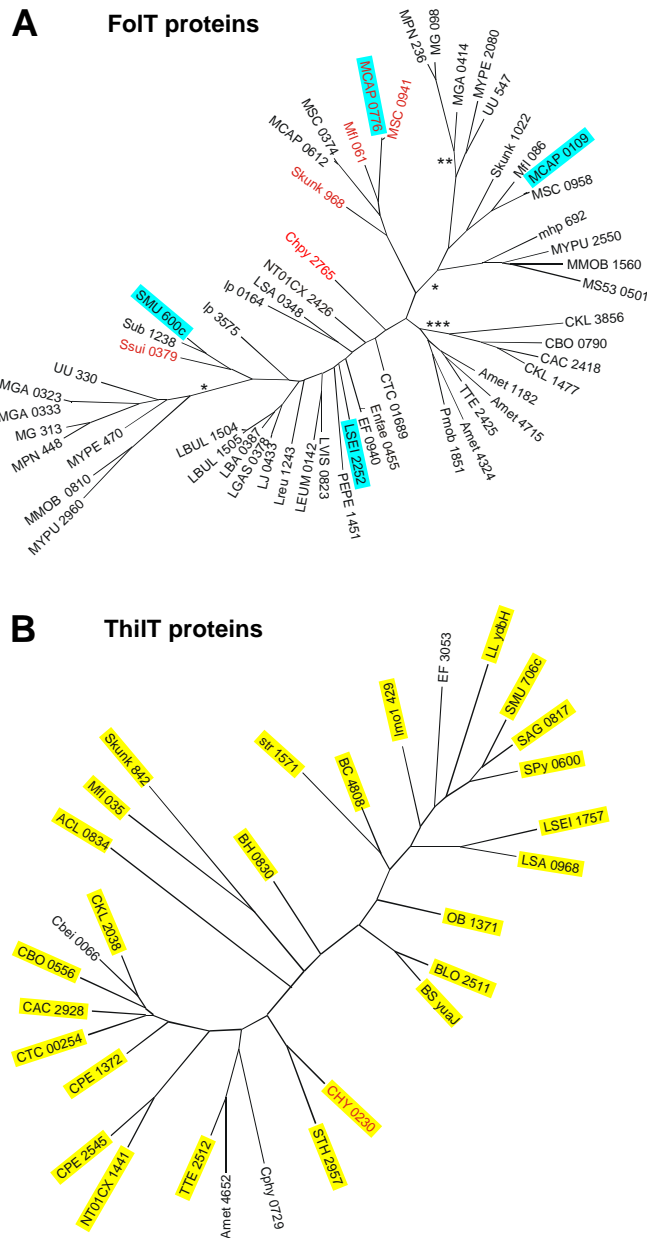
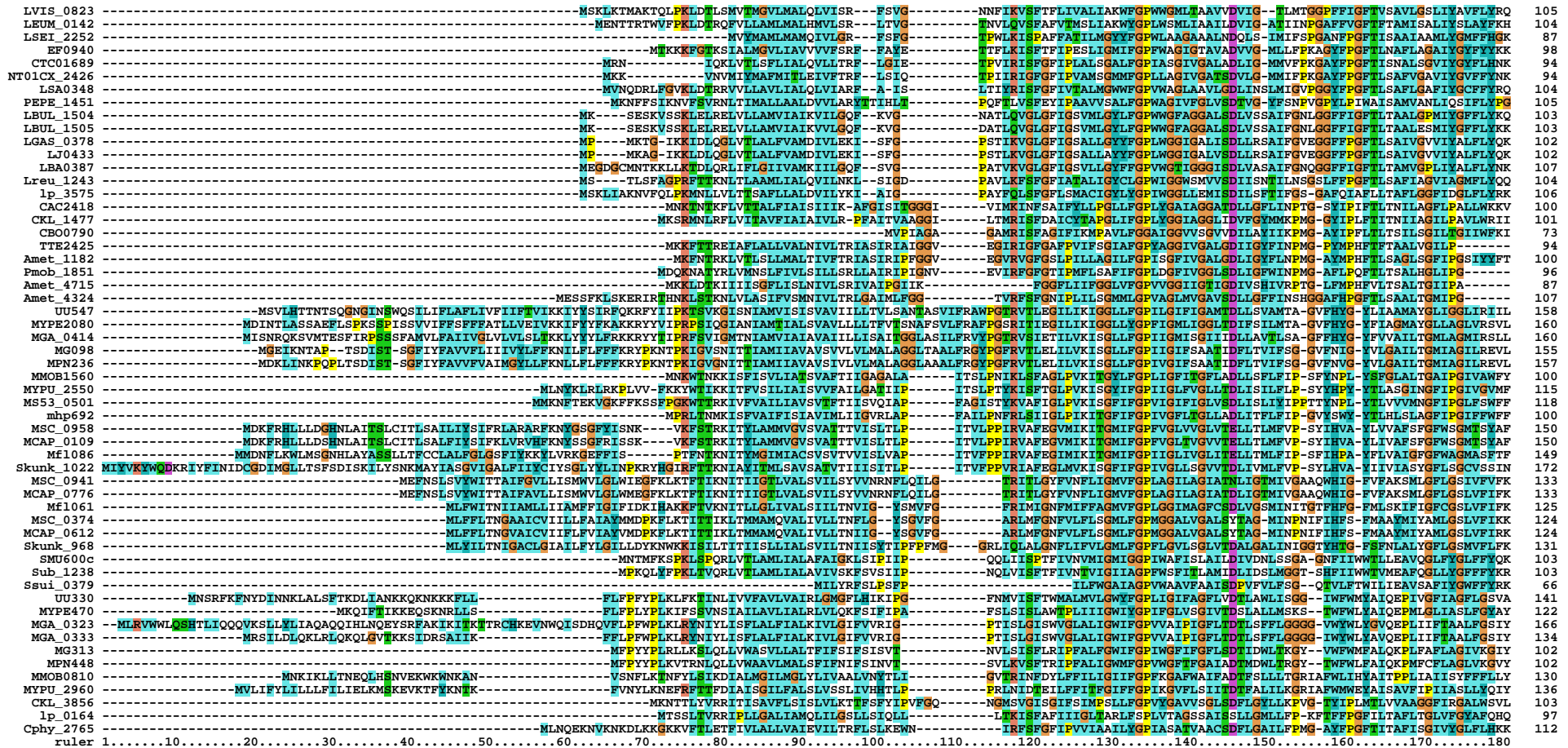


FIG. S1. Phylogenetic analysis of FolT and ThiT family proteins. After sequence alignment with ClustalW, the PHYLIP package was used to construct maximum likelihood trees, including bootstrapping with 100 replicates and drawing of a consensus tree. Genome abbreviations are listed in Supplemental Table 1. (A) Tree of FolT family proteins. The *folT* genes encoding proteins shown in red cluster with the folate salvage gene *folC*. Proteins highlighted in blue were shown in this study to bind folates. Asterisks indicate special features, as follows: *, two groups of *Mycoplasma* proteins with an insertion (~100-residues) containing two transmembrane domains after the third transmembrane domain; **, three *Mycoplasma* proteins with an additional hydrophilic C-terminal domain (~100 residues) similar to aspartyltRNA amidotransferase subunit C; ***, four *Clostridium* proteins with an insertion (~85 residues) containing two transmembrane domains after the third transmembrane domain. (B) Tree of ThiT family proteins. The *thiT* gene encoding the protein shown in red clusters with the thiamine salvage gene *thiN* (thiamine pyrophosphokinase). Proteins highlighted in yellow are encoded by *thiT* genes that are preceded by a thiamine pyrophosphate riboswitch.

Fig. S2. Sequence alignment of FolT proteins

Histograms below alignment show the degree of residue conservation at each position

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IVIS_0823 P VSWWRVIGA 133
 LEUM_0142 DK VSWWRVISA 133
 LSETI_2252 E VTLVRVILA 115
 EF0940 E MTQWRVILA 126
 CTC01689 K KIILNIALA 122
 NT01CX_2426 K VSVKRVILA 122
 LSA0348 K ITNLRVILA 132
 PEPE_1451 K TDWWRVILA 131
 LBUL_1504 P IQWRVIAS 133
 LBUL_1505 P IQWRVIAS 131
 LGAS_0378 P IKWWRVAA 130
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 LBA0387 P IKWWRIVVS 135
 Lreu_1243 R ISWQRILIV 130
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 Amet_4715 RINVRYPFKR RINVWSLAISGLG 137
 Amet_4324 IVVRSCLKIDL AKLSWSKIIIVSIFVG 131
 MIVMLSPKN KSMNVNVAANLAV 131
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 MYPE2080 SFA--KESRWYAIISIIAT ICSLAAI VFYISISTSGF-EQSIIFPIPIP-SSPDKISREFLSYIPGECVFWLLIMLWFFFS NRYQKWKMLYGNKKHKPIRPKKIDKHYPNNECL 294
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 mhp692 VIKGKWFEKKSSILIRLDQKIEFEQKQIFDFIVYRIANNQ KDENLERKMKQKLLFLQKKVVKVESWQBEKSLNLFYWIASNLILSIVTITIVVWFSSSIDFSQSRFIS SKLSFLIITLFGIVSMIIFLLARFINFERKNERVLTIAPIVPSALHEFASIIIGA 261
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 MCAP_0109 KLKKN-WLTLVFVVELLISA GIMFWLMGKM-EINPETSLEFGVPIADIVPFLFLIM ISITLIFIFYGLVLVI HIKKQKWLEVMPLPIILLCVIS EILVTVLVAAMGDQMLG 262
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 Mf1061 KNN-FWFKMIFLFLFSLAIS FISHPIAMLAIGKNAYVY FISHPIAMLAIGKNAYVY INYVKKIILPIELLIFI 182
 MSC_0374 QKSRLSFMISVEVLLFIASFILT FISHPVAMLAIGKNSVYV FISHPVAMLAIGKNSVYV VTLVKKFIIVFPDAVIEP 183
 MCAP_0612 QKSRLSFMISVEVLLFIASFILT FAENVLVLTIGLKQVLEP FAENVLVLTIGLKQVLEP VTLIKKFIIVFPDAVIEP 183
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 Sub_1238 FLRSNQKDD WLVVITATAIM WLVVITATAIM LIGSFIFTPLVQIYY 140
 Ssui_0379 PLDTKNKAD WLVVAGVVLVIQ WLVVAGVVLVIQ LFSFLITPLLIQIYE 140
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 MG313 LVRKNASNY-KVDFWVLQILVITFFVSVLULLLXLTNN- EFGQIGTKNEQTADLSVNVVVLQIITLVSVFVIFVLVEGLFGFVVKVRNKEQ- ALLTIXSLVLMVLS VVVSILLTGTAAIEFIF 226
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 Cphy_2765 Q- SLPNVIGA SLPNVIGA AVNQFFGLVINSVYLS 139
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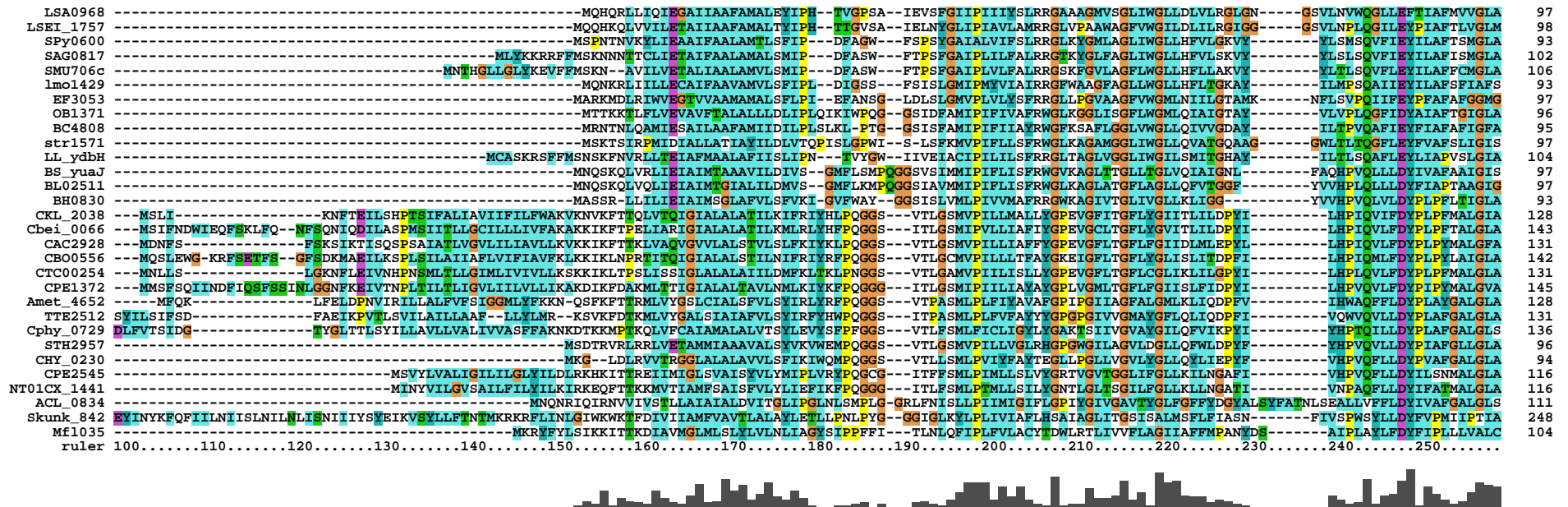
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CTC01689 ILTKKG--VYAITGLRIKLNIEVPKIVSCIVFVKLVG--VHLNKDIKNIAV----- 171
NT01CX_2426 ILTGKA--VKVLFTRLVKEAIMPPIHAIVYGAWKMDRLIEIMNKVAKFNK----- 172
LSA0348 ILSQTF--ISVHPATRAVGEAATMVTHPIVIVLVMHFEPVRLKDRIQ----- 178
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LBUL_1505 ML--GGTNFMVALSSRIKEMITPWIMVWVWFVILEGLSRVKLSRK----- 176
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Lreu_1243 LMSLSSSS--TGHTFMALLFIRLPKELITWPIETLLVLVLRLQSLRNLIITKNEK----- 186
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CB00790 SFMFLWIP--RIVEEVFMIVFESTAVSLLRLVYESVVLKISN----- 231
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Pmob_1851 SVMVLMPP--RIGGYVVSFFLNAPIMLLLRITPQINMKMEKK----- 178
Amet_4715 PLAVS----- 136
Amet_4324 AFLALLEF--RITSLIAGINIFPLTALVGLKKIPVFN----- 187
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Skunk_1022 VSQNKNVGT--DGYGVMTITRLAMAPIKVLFNSAIYIITVRAVSPLIHKDTNANLQY----- 340
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Sub_1238 --HVPFWAQYAAGRWPKIFEIPLRVLLTMPLIPPLQRIPEIKKLSAL----- 185
Ssu1_0379 --GTPWIVLVSSRLIKAVFEIPLRIVVTMLVPLSLQKIPELAKLMGK----- 149
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MG313 INNGRPSNNFVLYGVYFFLLPRVLVQALLPIYVALFYPLIGIVENNKNYLLFLTLSWKS----- 287
MPN448 LS-GKPSKNFVKYGSYFFLLPRVLVQALLMPLYLALFKPLIRIAENNLNRYLVNLSWKR----- 285
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MYPV_2960 FLPSKRVRLHKDLYFTWQGP--IIKSLFTIPTYSILVPLISVLNFEKQYIEKKQIMVQNQI----- 309
CKL_3856 VLRETIPTAWKVLPPFAVIWIPRVIEEILQNTVKAFVVLIMGICRKQNTLQEIMNGSSVKFK----- 300
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Cphy_2765 IISGKS--TFWGLLIPISIQSAVMSIIVISVTVVISKIIVPIIKKAIVIM----- 187
ruler .....370.....380.....390.....400.....410.....420.....430.....440.....450.....460.....470.....480.....490.....500.....510.....520.....530.....

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Fig. S3. Sequence alignment of ThiT proteins

Histograms below alignment show the degree of residue conservation at each position

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LSA0968	GVLQKP	-VQA	-----	SIQNTKVK	ITALLWSAAFIC	---TFAKYFCHFLAGAIYWG	GSYAPKG	-----	MNAWLYSLIVN	-GGSFVAGFILNGIVFGILLAMI	PKLYLPKD	-MFLKVO	SEK	200																
LSEI_1757	GLTFAS	-FOK	-----	AVRGSEKVKAS	GYAFAGIIG	---TFAKYFIHFIAGVVFVG	GAYAPKG	-----	INVWVYSLIVN	-GGSALFS	VLLIVVVGVLL	VAPQLFVAKD	GKSFSSTKAA	201																
SPY0600	GSFSDS	-LIK	-----	TLRRQQTFFAVFLAIMASLLA	---VTVRYLWHFLAGIIFWG	GSYAPKG	-----	MSAVWYFSFVN	-GTAGVLTFLIT	CLALMIAL	PIHPQLFDPKD	-----	187																	
SAG0817	GVFSAK	-FKD	-----	ALSSSSKTKALSIALSGAILA	---TFVRYVWHYIAGVIFWAS	YAPKG	-----	MSATLYSLSVN	-GTAGLLTLFFVVISI	IIILVISYPS	FFLPKK	-----	196																	
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EF3053	GVFARK	-IQL	-----	YFQANRLKSAIRTIILGSSVVA	---VFARFWHFHWAGVLVWGM	YAPKG	-----	MSPVLYSFVLN	-GSTVVNCLYVSLVGLLAK	VAPQLFVPKK	-----	191																		
OB1371	GVVAKS	-VOK	-----	NVRENKVKSYLSLITLGVFIG	---SFARFIAHYIAGIVVF	-ESAVEG	-----	QPVWLYSLLYN	-GSYMLPSFVLVSAVAVFF	LFHKQPKTL	LQTK	-----	189																	
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LL_ydbH	GLFRQK	-IAS	-----	-----	LKLVPVLLGTFVA	---VLLKYFFHFIAGIIFWS	QYAWKG	-----	WGAVAYSILAVN	-GISAILTATAAFVILII	FVKKPKLFIYSNY	-----	191																	
BS_yuaJ	GCFASS	-VRKA	AVS	-----	KTKGKLIVSVVS	AVFIG	---SLLRYAAHVISGAVVFG	SFAPKG	-----	TPVWIYSLIYN	-ATYMPVPSFIICAIVLC	LLFMTAPRL	LKSDKA	192																
BL02511	GFFAGS	-IRNAS	FSE	-----	KQKGKIISYVML	AVLLG	---SGLKFLAHVISGAVVFG	NYAPKG	-----	TPVWIYSLIYN	-GTMYLPSFIVCTIIVLC	ILLTAPRL	LENRTNMR	194																
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Cbei_0066	GFFKQR	-----	-----	-----	-----	RLVGVS	IA	---ILVRFLCHLIS	GIVFFG	SFAPKG	-----	MSPVVYSLIVN	-GPMIGIEGIIICLV	ILAVLPIERIF	HASSVNTISN	225														
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CTC00254	GYFRNN	-----	-----	-----	-----	KILAT	IVA	---IFGRFVHFHFI	AGVIFWG	STAPET	-----	MSPVLYSLIYN	-GSLFSVNALICV	VILYMLPVEN	LYKI	INRKS	HYNVS	215												
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TTE2512	GFFRKN	-----	-----	-----	-----	LSLGIL	VG	---GFCGRFFS	HFLSGI	FFFA	YAPKG	-----	MNPDIYSLLVN	-GSIICTEVLIC	FVVSLLIPQVR	NAIEV	K-KRALA	-----	212											
Cphy_0729	GLFYKSK-N	-----	-----	-----	-----	GLLKG	YLVG	---ITGRWLFS	ILSGFI	FFG	YAWEG	-----	WNPILYSMAYN	-GAYIYAEGIA	TVIIICLP	VKNADFQ	VKMT	YLD	-----	221										
STH2957	GLARGH	-----	-----	-----	-----	ARWAA	AWLGS	LAIA	GRFAAH	VIAGV	VFFG	QFAPKG	-----	QSPVLYSLIYN	-GSYLLPEAVLS	--AVLLM	VLLPAL	DRAL	PAQV	GS	SAH	182								
CHY_0230	GLFAKK	-----	-----	-----	-----	SPYP	PGVVL	---AILGRFLSH	LISG	VFFG	YAPKG	-----	QSPVWYSAIYN	-GSYLLPELIL	LGAVYLL	KIFER	LKVT	-----	171											
CPE2545	GSFGIDKKY	-----	-----	-----	-----	KIFLG	SLMA	---GSIS	TFVSIIS	GVIFFG	QYAPKG	-----	MNLWIYSIYN	-TSAGVSELL	TAIVITIMP	IKMLAK	RMK	FTNNI	-----	201										
NT01CX_1441	GTFGNDNKI	-----	-----	-----	-----	KIGLG	SLMA	---VVLVS	VFNVL	SGAVYFG	QYAPKG	-----	MNVWLYSFMYN	-ISYAGVE	GALCIIILLIL	PIKRL	KINK	-----	195											
ACL_0834	GFFKKA	-----	-----	-----	-----	LDSRI	WFSI	ITVVVLL	IRWLS	---IIVGAIL	WAAYAS	SEFTON	LMDN	QNAFIYS	GIYN	---IIYTL	TAL	SI	IGSLAL	POFKEL	KLQF	GSN	208							
Skunk_842	GFIRFKVN	-----	-----	-----	-----	NDKK	YITV	NYIIICFS	IIIL	ILYSQ	VLGVGLI	WITL	LF	PD	SV	WPG	---YSNWL	YAI	VNFIE	FLFTY	PIIQIV	VPFIL	RS	LAFIFW	ORYL	KY	DS	348		
Mf1035	SVFIP	PREYKQYLEKANVN	-----	-----	-----	LKFWD	RVKFN	VKRISL	WLNRW	VFIL	VIIIS	FLG	FWSK	TFAG	VL	FY	QYAPKG	-----	QGVWIYSMSVN	-AVNSV	FDLL	LLI	II	PIIC	TL	FL	KN	KIY	217	
ruler	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	...



Supplemental Table 1

Locus tags of FolT and ThiT proteins identified in various bacterial genomes

FolT proteins	
Organism	Locus tag
<i>Clostridium acetobutylicum</i> ATCC 824	CAC2418
<i>Clostridium botulinum</i> A str. ATCC 3502	CBO0790
<i>Clostridium kluyveri</i> DSM 555	CKL 3856
<i>Clostridium kluyveri</i> DSM 555	CKL 1477
<i>Clostridium novyi</i> NT	NT01CX_2426
<i>Clostridium phytofermentans</i> ISDg	Cphy_2765
<i>Clostridium tetani</i> E88	CTC01689
<i>Enterococcus faecium</i> DO	EFAE 0455
<i>Enterococcus faecalis</i> V583	EF0940
<i>Lactobacillus acidophilus</i> NCFM	LBA0387
<i>Lactobacillus brevis</i> ATCC 367	LVIS_0823
<i>Lactobacillus casei</i> ATCC 334	LSEI 2252
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> ATCC BAA-365	LBUL 1505
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> ATCC BAA-365	LBUL 1504
<i>Lactobacillus gasseri</i> ATCC 33323	LGAS 0378
<i>Lactobacillus johnsonii</i> NCC 533	LJ0433
<i>Lactobacillus plantarum</i> WCFS1	lp_3575
<i>Lactobacillus plantarum</i> WCFS1	lp_0164
<i>Lactobacillus reuteri</i> F275	LREU 1243
<i>Lactobacillus sakei</i> subsp. <i>sakei</i> 23K	LSA0348
<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i> ATCC 8293	LEUM_0142
<i>Mesoplasma florum</i> L1	MFL061
<i>Mesoplasma florum</i> L1	Mfl086
<i>Mycoplasma capricolum</i> subsp. <i>capricolum</i> ATCC 27343	MCAP_0612
<i>Mycoplasma capricolum</i> subsp. <i>capricolum</i> ATCC 27343	MCAP_0776
<i>Mycoplasma capricolum</i> subsp. <i>capricolum</i> ATCC 27343	MCAP_0109
<i>Mycoplasma gallisepticum</i> R	MGA 0333
<i>Mycoplasma gallisepticum</i> R	MGA 0323
<i>Mycoplasma gallisepticum</i> R	MGA 0414
<i>Mycoplasma genitalium</i> G37	MG_313
<i>Mycoplasma genitalium</i> G37	MG_098
<i>Mycoplasma hyopneumoniae</i> 232	MHP692
<i>Mycoplasma mobile</i> 163K	MMOB0810
<i>Mycoplasma mobile</i> 163K	MMOB1560
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> SC str. PG1	MSC 0374
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> SC str. PG1	MSC 0941
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> SC str. PG1	MSC 0958
<i>Mycoplasma penetrans</i> HF2	MYPE470
<i>Mycoplasma penetrans</i> HF2	MYPE2080
<i>Mycoplasma pneumoniae</i> M129	MPN448
<i>Mycoplasma pneumoniae</i> M129	MPN236
<i>Mycoplasma pulmonis</i> UAB CTIP	MYPU 2960
<i>Mycoplasma pulmonis</i> UAB CTIP	MYPU 2550
<i>Mycoplasma synoviae</i> 53	MS53 0501
<i>Petrotoga mobilis</i> SJ95	PMOB 1851
<i>Pediococcus pentosaceus</i> ATCC 25745	PEPE 1451
<i>Spiroplasma kunkelii</i> CR2-3X	Skunk_968
<i>Spiroplasma kunkelii</i> CR2-3X	Skunk_1022
<i>Streptococcus mutans</i> UA159	SMU.600c
<i>Streptococcus suis</i> 89/1591	Ssui_0379
<i>Streptococcus uberis</i> 0140J	SUB1238
<i>Ureaplasma parvum</i> serovar 3 ATCC 700970	UU330
<i>Ureaplasma parvum</i> serovar 3 ATCC 700970	UU547

ThiT proteins

Organism	Locus tag
<i>Acholeplasma laidlawii</i> PG-8A	ACL_0834
<i>Alkaliphilus metalliredigens</i> QYMF	Amet_4652
<i>Bacillus halodurans</i> C-125	BH0830
<i>Bacillus cereus</i> ATCC 14579	BC4808
<i>Bacillus licheniformis</i> ATCC 14580	BL02511
<i>Bacillus subtilis subsp. subtilis</i> str. 168	BSU30990
<i>Carboxydotherrnus hydrogenoformans</i> Z-2901	CHY_0230
<i>Clostridium acetobutylicum</i> ATCC 824	CAC2928
<i>Clostridium beijerinckii</i> NCIMB 8052	Cbei_0066
<i>Clostridium botulinum</i> A str. ATCC 3502	CBO0556
<i>Clostridium kluyveri</i> DSM 555	CKL_2038
<i>Clostridium perfringens</i> str. 13	CPE2545
<i>Clostridium perfringens</i> str. 13	CPE1372
<i>Clostridium phytofermentans</i> ISDg	Cphy_0729
<i>Clostridium tetani</i> E88	CTC00254
<i>Enterococcus faecalis</i> V583	EF3053
<i>Lactobacillus casei</i> ATCC 334	LSEI_1757
<i>Lactococcus lactis subsp. lactis</i> II1403	L116212
<i>Listeria monocytogenes</i> EGD-e	lmo1429
<i>Oceanobacillus iheyensis</i> HTE831	OB1371
<i>Spiroplasma kunkelii</i> CR2-3X	Skunk_842
<i>Streptococcus agalactiae</i> 2603V/R	SAG0817
<i>Streptococcus mutans</i> UA159	SMU.706c
<i>Streptococcus pyogenes</i> M1 GAS	YP_602012
<i>Streptococcus thermophilus</i> CNRZ1066	str1571
<i>Symbiobacterium thermophilum</i> IAM 14863	STH2957
<i>Thermoanaerobacter tengcongensis</i> MB4	TTE2512